

Effect of Operating Condition on Performance of Anaerobic Hybrid Reactor at Thermophilic Temperature

Sorawit Wanitanukul¹, Warin Rukruem² and Pawinee Chaiprasert^{3*}

¹Energy Technology and Management, The Joint Graduate School of Energy and Environment,
King Mongkut's University of Technology Thonburi, Bangmod, Bangkok 10140, Thailand

²Excellent Center of Waste Utilization and Management, Pilot Plant Development and Training Institute,
King Mongkut's University of Technology Thonburi, Bangkokthian, Bangkok 10150, Thailand

³Biotechnology Program, School of Bioresources and Technology, King Mongkut's University of Technology
Thonburi, Bangkokthian, Bangkok 10150, Thailand

*Corresponding author: pawinee.cha@kmutt.ac.th

Abstract

Anaerobic hybrid reactor (AHR), like the upflow anaerobic sludge bed-fixed film reactor, was applied to treat and produce methane from raw palm oil mill effluent (POME) with high chemical oxygen demand (COD) and suspended solid (SS). This study was aimed at improving anaerobically digested POME at thermophilic temperature (55 °C) through optimization of organic loading rate (OLR) and hydraulic retention time (HRT). COD removal and methane production were measured as indicators of the reactor performance as well as pH and ratio of total volatile acid (TVA) and alkalinity (Alk) were used for the reactor stability. Microbial activities of inoculum seed for acidogens and methanogens at thermophilic temperature were 0.17 gCOD_{methane}/gVSS.d and 0.72 gCOD_{glucose}/gVSS.d, respectively. Experimental results within 78 days showing that OLR and HRT of AHR for methane production increased from 1.2 to 5.5 gCOD/l.d and shortened from 20 to 10 d, respectively. AHR was investigated in order to achieve high reactor performance and stability. High microbial activities of acidogens and methanogens in sludge and packed zones of AHR were found during operation. High performances of COD removal efficiency and methane production rate were in the range of 80-90% and increased from 1.35 to 7.82 l/d, respectively, when the OLR increased and HRT decreased within the tested range. In addition, methane yield coefficient was increased from 0.19 to 0.32 lCH₄/gCOD removed. High reactor stability could keep in pH 7.0-8.0 and TVA/Alk was lower than 0.5.

Keywords: Anaerobic hybrid reactor, Palm oil mill effluent, Operating condition, Thermophilic temperature.

1. Introduction

The world's demand for energy is growing continuously. An alternative source of energy is coming interested. One of the interesting of renewable energy is the energy from biowaste, one of them is the energy production from palm oil waste. Palm oil is an importance source for food production and biodiesel, which is a renewable energy, production that can be use instant the conventional diesel fuel. Palm oil is providing 43.1 million tones or 27% of the world's total edible oil and fat production, followed by soybean oil (Chiew et al., 2011). Oil palm plantations in Thailand are expanding year by year. There are large amount of waste that generated from a process of oil palm production. Palm oil mill effluent (POME), which is polluting wastewater and could not discharged directly to the environment, is generated from the palm oil production process. Typically, 1 ton of crude palm oil production generated POME approximately 5.0-7.5 m³ (Somporn et al., 2004). POME is a viscous brown liquid and contained of suspended solid at pH ranges between 4 and 5. A characteristic of POME has high COD, BOD and suspended solid by approximately 90,000, 30,000 and 25,000 mg/l, respectively and POME was discharged at high temperature (80-90 °C) (Mustapha et al., 2003; Najafpour et al., 2006; Meesap et al., 2012). POME was shared the potential of biogas production with the total potential of the biogas production from the major agro-industrial waste in Thailand by 8.82% or 88.6 million m³/year (Chaiprasert, 2011).

Environmental impacts from wastewater of palm oil production known as POME are a matter of great concern. POME contains large quantities of high organic pollutants and classified as high strength wastewater. Biological treatment of POME is the most frequently used treatment method. Since it contains high concentrations of organic matter, adoption of anaerobic digestion (AD) in the first stage of the process is needed to convert the bulk of the POME to biomethane as renewable energy as well as treatment of POME in the same time (Poh and Chong, 2009; Meesap et al., 2012). An anaerobic reactor have been studied in laboratory scaled for POME treatment such as up-flow anaerobic sludge blanket (UASB) reactor, up-flow anaerobic filtration, fluidized bed

reactor, and up-flow anaerobic sludge bed-fixedfilm (AHR). The AHR, high rate anaerobic bioreactor, was found to be high performing in COD removal efficiency and methane production (Pohand Chong, 2009; Meesap et al., 2012). Upflow AHRs can work well for the high-suspended solid pollutants like cassava starch wastewater, slaughterhouse waste, and POME (Borja, et al., 1998; Chaiprasert et al., 2003; Najafpour et al., 2006; Zinatizadeh et al., 2007; Meesap et al., 2012). Therefore, this study applied AHR for the anaerobic treatment of POME.

The major components in biological anaerobic digestion, not only microorganisms play an important role as the main function in controlling reactor performance and stability but the operational and environmental parameters of the process also obviously affect the microbial behavior resulting in wastewater treatment and biogas production performances. Operating temperature is one of importance factor that influence to the digester performance and stability. Normally, the mesophilic condition (below 45°C) has been widely used for wastewater treatment. Thermophilic anaerobic digestion of POME has been tried previously since it would be advantageous to carry out the anaerobic digestion under thermophilic conditions within the temperature range of 49-57 °C (Yang et al., 2008; Ahn et al., 2000; Yu et al., 2002) with the POME temperature in sump pond varying between 45 and 70 °C. It is generally recognized that thermophilic operation has the potential for a faster bacterial growth and consequently higher treatment rates (Khemkhao et al., 2011). Therefore, in this study thermophilic temperature (55°C) was used for POME treatment using anaerobic hybrid reactor (AHR), which content sludge zone (part of microbial sludge) and packed zone (part of the microbial biofilm on the packing material). The performance and stability of the AHR at various organic loading rate (OLR) and hydraulic retention time (HRT) were measured in term of pH, Total Volatile Acid (TVA), Alkalinity (Alk), COD efficiency removal, biogas and methane production, and their yield.

2. Method

2.1 Characteristics of wastewater and inoculum seed

Raw POME was collected from palm oil production plant at Chonburi province, Thailand. The characteristics of raw POME were determined pH, chemical oxygen demand (COD), oil and grease (O&G), total solid (TS), total suspended solid (TSS), volatile suspended solid (VSS), volatile fatty acid (TVA), and alkalinity (Alk). POME characteristics were determined according to the procedures of the standard methods of wastewater analysis (APHA, 2005). Its characteristics were shown in Table 1. High in COD (91,800 mg/l), TS (65,845 mg/l), SS (36,470 mg/l), volatile solid in form of TS and SS, and oil and grease (O&G).

Table 1. Characteristics of raw POME

Parameters	Value
pH	4.26
Alk (mg/l)	1,195
TVA (mg/l)	4,935
TCOD (mg/l)	91,800
TS (mg/l)	65,845
SS (mg/l)	36,470
TVS (mg/l)	57,720
VSS (mg/l)	23,240
O&G (mg/l)	19,320

The inoculum seed was taken from the anaerobic wastewater treatment system of POME at mesophilic condition. The inoculum seed was acclimatized to thermophilic temperature at 55 °C. The characteristics of seed were monitored in term of TS, SS, VS, activity for acidogens and methanogens were monitored. Glucose and acetate were used as the substrates for activity analysis of the acidogens and methanogens, respectively. Specific glucose utilization (SGU) and methanogenic activity (SMA) at 55 °C were calculated. Determination of microbial activity was performed using the method of Nopharatana et al. (1998). High concentration of biomass (VSS) in inoculum seed (77% SS) with activities of acidogens (SGU) and methanogens (SMA) were found at value of were 0.72 gCODglucose/gVSS.d and 0.17 gCODmethane/gVSS.d, respectively (Table 2).

Table 2. Characteristics of inoculums seed

Parameters	Value
TS (mg/l)	33,210
SS (mg/l)	26,100
TVS (mg/l)	21,050
VSS (mg/l)	20,085
SGU (gCODglucose/gVSS.d)	0.72
SMA (gCODmethane/gVSS.d)	0.17

2.2 Study of AHR performance and stability at various OLR and HRT

2.2.1 Reactor set up

The laboratory anaerobic hybrid reactor (AHR) system was shown in a schematic diagram of Figure 1. AHR is made from acrylic column with a diameter of 9.6 cm. and a height 90 cm with total volume of 6,000 ml. The working volume of the reactor is 5,800 ml. The bottom half and the upper half of reactor working volume was occupied by sludge zone and packed zone, respectively. The packed zone was contained nylon fibers, which there are the specific surface area $2 \text{ m}^2/\text{m}^3$, was installed to the reactors with density of $30 \text{ kg}/\text{m}^3$ for microbial attachment as biofilm formation. A silicone tube was wound around the reactors for controlling of the reactors temperature at 55°C by water bath. An insulator was covered around the reactors. Three sampling ports were distributed at several height of AHR. The POME treatment system was contained of AHR, influent and effluent tank, peristaltic pump, gas counter and water bath (Figure 1).

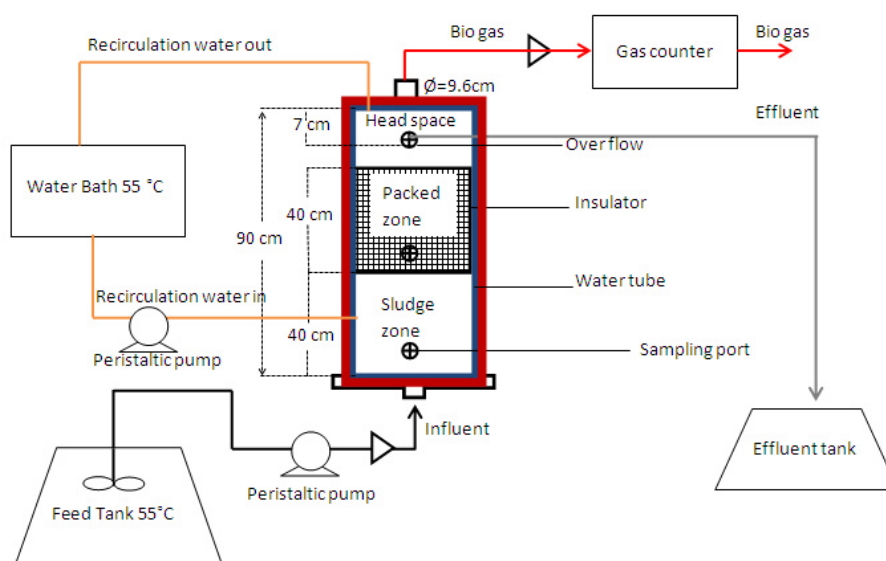


Figure 1. Schematic diagram of the AHR system

2.2.2 Reactor start-up and operation

The reactor was operated under temperature 55°C as a thermophilic condition. Raw POME was semi-continuously fed upflow from the bottom of sludge bed zone up to the packed zone. The start-up of reactor was initially fed POME at OLR of $1.2 \text{ gTCOD}/\text{l.d}$ with HRT of 20 d. Upon reactor start-up operation, the experiments were set up by step of increasing OLR and shorter HRT. The operating condition in this study was shown in Table 3. The reactor performance, stability, and microbial activity were monitored and analyzed. The process performance of the overall AHR, was routinely monitored through measurement of the TCOD, TVA, Alk and pH by followed the procedures of the standard methods of wastewater analysis (APHA, 2005). The pH, TVA, Alk and biogas production were determined daily. TCOD was measured two times a week. Biogas production

was measured by gas counter with water replacement and biogas composition was determined using gas chromatography (Panichnumsin et al., 2010).

Table 3. Operating condition at various OLR and HRT

Operating period (d)	OLR (gCOD/l.d)	HRT (d)
1-12	1.2	20
13-18	2.0	15
19-28	2.6	15
29-38	2.6	10
39-53	3.5	10
54-66	4.5	10
67-78	5.5	10

2.2.3 Microbial activity monitoring

Determination of microbial activity was carried out in triplicate using 120 mL vials with 100 mL of working volume. The substrate (F)-inoculum (M) ratio (F/M) in the final volume was 0.1gCOD/gVSS. Glucose and acetate were used as the substrates for activity analysis of the acidogens (SGU) and methanogens (SMA), respectively. Determination of microbial activity was performed using the method of Nopharatana et al., 1998. The microbial activities of the sludge and packed zones in AHR, the suspended sludge samples from the sludge zone and the attached biofilm samples from the supporting media in the packed zone were collected and determined SS, VSS and microbial activities at the OLR of 3.5 and 5.5 gCOD/l.d.

3. Results and discussion

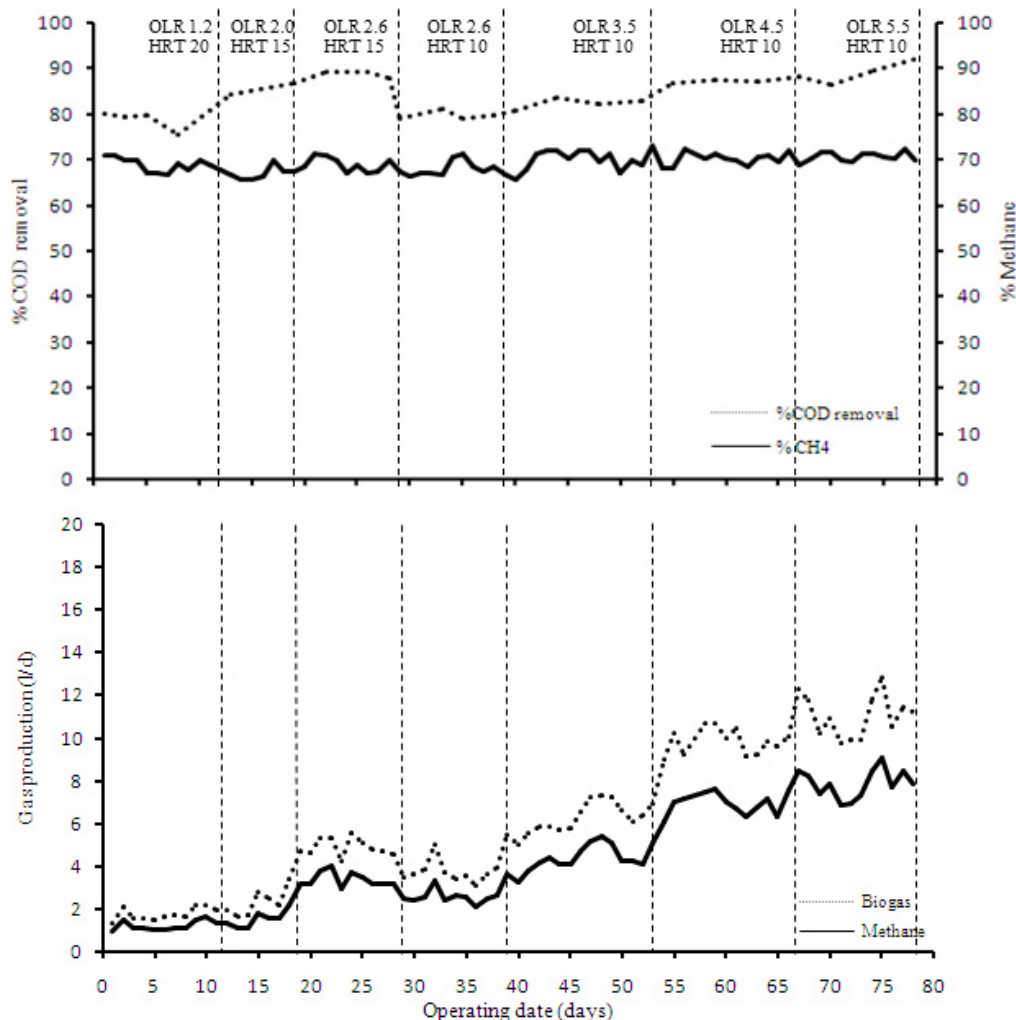
The AHR was operated for 80 days consecutively by increasing the OLR and decreased HRT as shown in Table 3. The operating dates for each condition were done in the short period to fasten the step up of the higher OLR and shorter HRT. Therefore, the determination of the flexibility and capability of AHR to treat and produce methane from POME at various OLR and HRT were studied on reactor performance and stability. The reactor was operated by increasing OLR from 1.2 gCOD/l.d to 5.5 gCOD/l.d and decreasing HRT from 20 d to 10 d at high temperature condition (thermophilic 55°C). Raw POME was fed into the reactor; start with COD concentration of 24,000 mg/l (OLR 1.2 gCOD/l.d at 20 d HRT) and increased stepwise to 55,000 mg/l (OLR 5.5 gCOD/l.d at 10 d HRT). Seven operating conditions of AHR at OLR 1.2 gCOD/l.d with 20 d HRT (OLR 1.2 HRT 20), OLR 2.0 gCOD/l.d with 15 d HRT (OLR 2.0 HRT 15), OLR 2.6 gCOD/l.d with 15 d HRT (OLR 2.6 HRT 15), OLR 2.6 gCOD/l.d with 10 d HRT (OLR 2.6 HRT 10), OLR 3.5 gCOD/l.d with 10 d HRT (OLR 3.5 HRT 10), OLR 4.5 gCOD/l.d with 10 d HRT (OLR 4.5 HRT 10), and OLR 5.5 gCOD/l.d with 10 d HRT (OLR 5.5 HRT 10) were carried out for 12, 6, 10, 10, 15, 13 and 12 d, respectively. The AHR performance and stability were shown in Figures 2 and 3, respectively.

3.1 Reactor performance

A successful operation of the AHR at high temperature was achieved up to 5.5 gCOD/l.d of OLR and 10 d of HRT. Effect of operating condition on reactor performance was shown in Figure 2 (a-b) and Table 4. Figure 2a was shown the percentage of COD removal and methane content from anaerobic digestion of POME in the AHR at each operating condition. Not much significant difference in COD removal and methane content among seven operating conditions. The efficiency of COD removed was achieved in the range of 80 – 90%. At beginning, the efficiency of COD removal was fluctuated when shorten HRT from 15 d to 10 d as can notice at the condition of OLR 2.6 gCOD/l.d. The decrease of HRT will shorten contact time of POME and microbial cells that effected on microbial degradation in POME and it might be the low cell concentration at initial stage of operation. After that the operating condition was step up increased OLR at constant HRT at 10 d, the efficiency of COD removal was gradually increased (seen in Figure 4). It can be noted that the AHR could be operated with these condition with high performance and the AHR could be operated with higher COD concentration condition.

Figure 2b was shown the performance of the AHR in biogas and methane production at different operating condition. It was found that the biogas and methane production were closely correlated with the increasing of OLR. The increasing of OLR from 1.2 to 5.5 gCOD/l.d, the biogas and methane production was increased from 1.32 to 11.18 l/d and 1.35 to 7.82 l/d, respectively. The content of methane during the operating condition was

found in the range of 65 – 73% (Figure 2a). The methane yield coefficient is defined as the ratio of methane produced in this experiment to the COD utilized. The methane yield increased corresponding to the increase of the OLR and the time of AHR operation. During the initial operation of AHR, a low methane yield was obtained, which was similar to the result found by Chaiprasert et al. (2003) and Poh and Chong (2009), likely due to the



organic carbon (COD) being consumed by microorganisms to build more cells during the initial startup period. Methane yield obtained at $0.32 \text{ lCH}_4/\text{gCOD}_{\text{removed}}$, which is the highest yield in this study. The efficiency of COD removed and methane yield at the end of each operating condition of this study were shown in Table 4. This value was close to the theoretical methane yield of $0.35 \text{ l H}_4/\text{gCOD}_{\text{removed}}$. Considering a theoretical methane yield, the whole of the organic matter is transformed into methane, accounting for virtually negligible biomass growth and cell maintenance (Borja et al., 2003).

3.2 Reactor stability

The high performance in term of COD removal, methane production and methane yield were found during the operation condition for 80 d. This phenomenon can be occurred it depends on the process stability that control by the environmental condition inside of reactor. The process stability in the reactor was investigated. The results were shown in Figure 3 (a-b). The reactor stability was achieved in pH constant with 7.0 – 8.0 (Figure 3a) and concentrations of TVA and Alk (Figure 3b) were increased when OLR was increased. The increasing of OLR was effected to TVA by at high concentration of COD, high organic matters were hydrolyzed to organic acid and cause of organic acid accumulation from the increasing of OLR. That mean, at high OLR condition the seed was hydrolyzed more organic matters for produced more organic acids. Along the operation, the concentrations of TVA and Alk were in the range of 1,000-1,500 mg $\text{CH}_3\text{COOH}/\text{l}$ and 2,000-3,000 mg CaCO_3/l , respectively. However, at the operating condition OLR 1.2 – 5.5 gCOD/l.d, TVA/Alk ratio was lower than 0.5 (Figure 3a), which reactor was operated under good environment condition. At this point, the environmental condition in the

anaerobic digestion process can control the system to neutralize the pH with a high buffer capacity and less acidification risk leading to the high process stability (Grau et al., 1975).

Figure 2. COD removal (a), methane content (a), biogas and methane production (b) of AHR performance at various operating condition

Table 4. COD removed and methane yield at the end of each operating condition

OLR (gCOD/l.d)	HRT (Days)	COD removal (%)	Methane yield (ICH ₄ /gCOD _{removed})
1.2	20	75.48	0.21
2.0	15	84.30	0.21
2.6	15	87.84	0.19
2.6	10	79.72	0.23
3.5	10	82.78	0.29
4.5	10	87.11	0.30
5.5	10	91.91	0.32

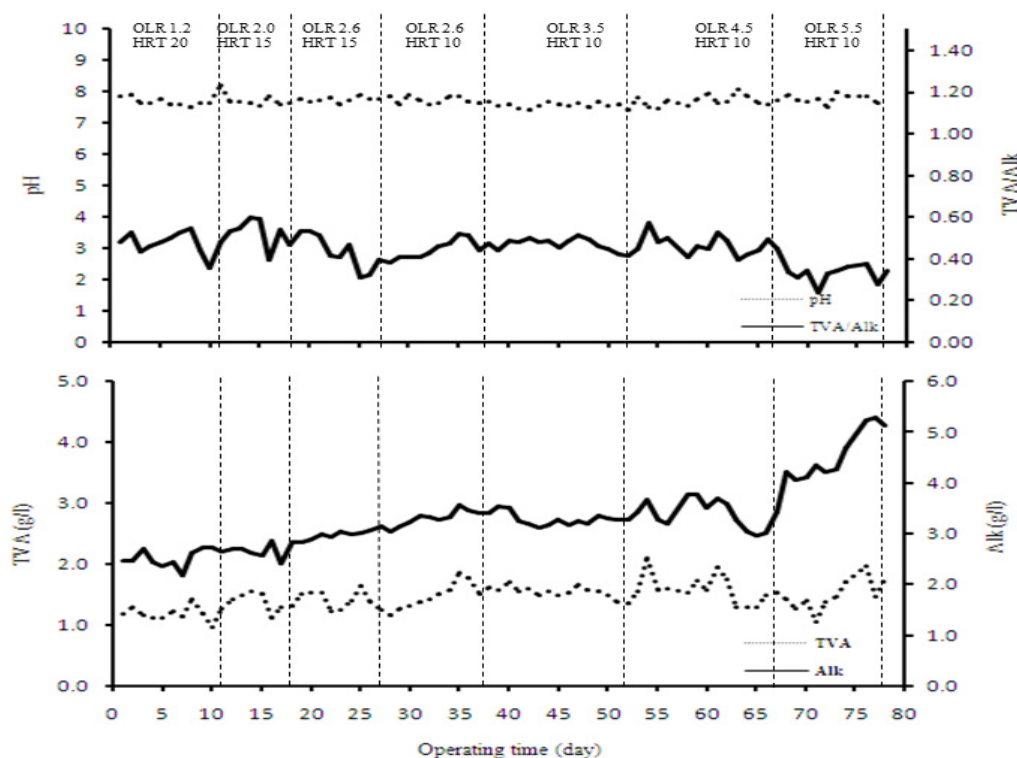


Figure 3. AHR stability in term of pH (a), TVA/Alk ratio (a), concentrations of TVA and Alk (b) at various operating condition

3.3 Acidogenic and methanogenic activities

The seeds were collected from Chonburi province, which operated in mesophilic anaerobic system and acclimatized seed to 55 °C. Then applied this inoculum seed in concentration of 20 gVSS/l to 6 l of AHR. The cell biomass in sludge and packed zone were monitored at operating condition OLR 3.5 HRT 10 and OLR 5.5 HRT 10 (Figure 4). The cell biomass concentration was more increased in sludge zone while that in packed was slightly constant when increased OLR 3.5 to 5.5 gVSS/l.d. However, total cell biomass was increased from 20.10 (initial seed) to 43.29 and 73.14 at OLR 3.5 and 5.5 gVSS/l.d, respectively. The cell biomass related to amount of substrate as food and increased the reactor performance in POME treatment and methane production.

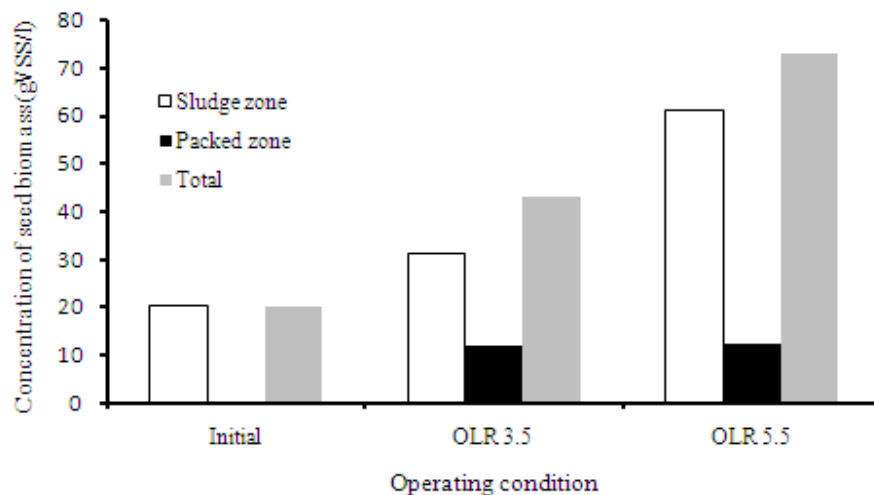


Figure 4. Cell biomass concentration in sludge and packed zones of AHR at OLR 3.5 and 5.5 gCOD/l.d

Not only the quantity of microbial cell will affect reactor performance but the microbial quality in term of microbial activity also was important. Microbial activities for acidogens and methanogens at operating condition OLR 3.5 HRT 10 and OLR 5.5 HRT 10 were shown in Table 5. At operating condition of OLR 3.5 HRT 10, acidogenic activity increased and methanogens slightly decreased compared to that in the startup seed. When the organic pollutant concentration was increased from the OLR 3.5 to the OLR 5.5 conditions, the activities of the acidogens and methanogens in the sludge and packed zones were investigated for the development of these characteristics. In the sludge zone, the acidogenic activity was significantly increased from 2.48 to 3.32 g COD_{glucose}/g VSS.d, while the methanogenic activity was slightly decreased from 0.14 to 0.12 g COD_{CH₄}/gVSS.d. In the packed zone, the acidogenic activity was significantly increased from 2.33 to 3.11 g COD_{glucose}/g VSS.d, while the methanogenic activity was slightly constant around 0.18-0.19 g COD_{CH₄}/gVSS.d. In addition, when comparing microbial activities between the packed zone and the sludge zone it was found that the acidogenic activity in packed zone was slightly lower whereas the methanogenic activity were slightly higher than sludge zone.

The sludge zone is the bottom part of the AHR, where there is the first contact with the influent POME. In this zone, most of the organic carbon was hydrolyzed to a simpler molecule and then converted to volatile fatty acids by acidogens. The packed zone was located in the top part of the AHR, where the remaining organic compounds were continuously converted to short chains of volatile fatty acids. This was reflected in the slightly lower activity of acidogens, while the methanogens characteristics were evident slightly higher in this packed zone.

The action of the sludge and packed zones, which work as hydrolysis/ fermentative and methanogenesis zones were mostly responsible for a properly enhanced reactor performance and maintained the process stability of the AHR. For POME, anaerobic digestion could be achieved completely within one reactor of an anaerobic hybrid reactor.

Table 5. Microbial activities of SGU and SMA in sludge and packed zones of AHR

Operating condition	SGU (gCOD _{glucose} /gVSS.d)		SMA (gCOD _{methane} /gVSS.d)	
	Sludge zone	Packed zone	Sludge zone	Packed zone
Initial 55°C	0.72	-	0.17	-
OLR 3.5 gCOD/l.d	2.48	2.33	0.14	0.19
OLR 5.5 gCOD/l.d	3.32	3.11	0.12	0.18

This AHR with 50% of suspended growth and 50% of attached growth by volume can load high proportions of organic matter up to 5.5 g COD/l.d. The results showed that the AHR was achieved high performance and stability in good environment condition at high temperature (55 °C), furthermore the AHR could be operated at higher OLR than 5.5 g COD/l.d and shorter HRT than 10 d. The process performance and stability with organic loading (OLR 5.5 g COD/l.d) fed to a high-rate anaerobic hybrid reactor for POME in this study were close to that in the study of Meesap et al., 2012 at mesophilic temperature (30-35 °C).

4. Conclusions

Anaerobic hybrid reactor (AHR) was operated under thermophilic condition (55°C), the OLR was increase from 1.2 to 5.5 gCOD/l.d, and HRT was reduced from 20 days to 10 days. The inoculum seed was obtained from

mesophilic anaerobic system and acclimatized to thermophilic temperature by step increasing 5 °C/day until to 55 °C in AHR. The stability of the AHR, which increased OLR and reduced HRT, was stable with pH 7.0-8.0, and TVA/Alk was lower than 0.5. High performance in COD removal (90%) and methane production (7.82 l/d) with high methane yield (0.32 gCH₄/gCOD_{removal}) were found. High AHR performance and stable system, it was due to the balance of microbial consortium between acetogens and methanogens in sludge and packed zones. Microbial activities of acetogens and methanogens during operation comparing to inoculum seed were increased from 0.7 to 3 gCOD_{methane}/gVSS.d and 0.17 to 0.19 gCOD_{glucose}/gVSS.d, respectively. The process performance and stability, as well as the microbial characteristics, varied according to the organic pollutant concentrations (OLR) and HRT. The AHR applied in the study can handle the OLR to 5.5 g COD/l.d at HRT 10 d with high performance and stability. The capability of this AHR illustrates that it can be uploaded more OLR and reduce HRT for the further study at thermophilic temperature.

Acknowledgements

I would like to express my gratitude for the partial master scholarship from Joint Graduate School of Energy and Environment (JGSEE) and research funding is supported by Biohythane project, Agricultural Research Development Agency (ARDA). The research facility was supported by Excellent Center of Waste Utilization and Management (ECoWaste), King Mongkut's University of Technology Thonburi.

References

- Ahn, J.H. and Foster, C.F., (2000), *A comparison of mesophilic and thermophilic anaerobic upflow filters*. Bioresource Technology, 73(3): p.201-205.
- APHA, (2005), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA.
- Borja, R., Banks C.J., Wang, Z. and Mancha, A., (1998) "Anaerobic digestion of slaughterhouse wastewater using a combination sludge blanket and filter arrangement in a single reactor. Bioresource Technology, 65(1-2): p.125-133.
- Borja, R., Mart, A., Rincon, B. and Raposo, F., (2003), *Kinetics for substrate utilization and methane production during thermophilic anaerobic digestion of two phases olive pomace (TPOP)*. Journal of Agricultural and Food Chemistry, 51(11): p. 3390-3395.
- Chaiprasert, P., Suvajittanont, W., Suraraksa, B., Tanticharoen, M. and Bhumiratana, S., (2003), *Nylon fibers as supporting media in anaerobic hybrid reactors: its effects on system's performance and microbial distribution*. Water Research, 37(19): p.4605-461.
- Chaiprasert P., (2011), *Biogas production from agricultural wastes in Thailand*. J Sustainable Energy & Environment (Special Issue): p.63-65.
- Grau, P., Dohanyos, M. and Chudoba, J., (1975), *Kinetics of multicomponent substrate removal by activated sludge*. Water Research, 9(7): p. 637-642.
- Khemkhao, M., Nuntakumjorn, B., Techkarnjanaruk, S. and Phalakornkule, C., (2011), *Effect of chitosan on UASB treating POME during a transition from mesophilic to thermophilic conditions*. Bioresource Technology, 102(7): p.4674-4681.
- Meesap, K., Techkarnjanaruk, S., Boonapatcharoen N. and Chaiprasert, P., (2012), *Microbial communities and their performances in anaerobic hybrid sludge bed-fixed film reactor for treatment of palm oil mill effluent under various organic pollutant concentration*. Journal of Biomedicine and Biotechnology, Article ID 902707, 11 p.
- Mustapha S., Ashhuby B., Rashid M., Azni I., (2003), *Start-up strategy of a thermophilic upflow anaerobic filter for treating palm oil mill effluent*. Process Safety and Environmental Protection, 81(4): p.262-266.
- Najafpour G.D., Zinatizadeh A.A.L., Mohamed A.R., Hasnain M. Isa, Nasrollahzadeh H., (2006), *High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor*. Process Biochemistry, 41: p.370-379.
- Noparatana, A., Clarke, W.P., Pullammanappallil, P.C., Silvey, P. and Chynoweth, D.P., (1998), *Evaluation of methanogenic activities during anaerobic digestion of municipal solid waste*. Bioresource Technology, 64(3): p.169-174.
- Panichnumsin, P., Noparatana, A., Ahring, B. and Chaiprasert, P., (2010), *Production of methane by co-digestion of cassava pulp with various concentrations of pig manure*. Biomass and Bioenergy, 34(8): p.1117-1124.
- Poh, P.E. and Chong M.F., (2009), *Development of anaerobic digestion method for palm oil mill effluent (POME) treatment*. Bioresource Technology, 100(1): p.1-9.
- Somporn P., Shabbir H. Gheewala., Savitri G., (2004), *Environmental evaluation of biodiesel production from palm oil in a life cycle perspective*. Thai National News Bureau Public Relation Department Thailand.

- Yang, Tsukahara Y.K., and Sawayama S., (2008), *Biodegradation and methane production from glycerol-containing synthetic waste with fixed-bed bioreactor under mesophilic and thermophilic anaerobic conditions* Process Biochemistry, 43(4): p.362-367.
- Yu, H.-Q., Fang H.H.D., and GC G-W., (2002), *Comparative performance of mesophilic and thermophilic acidogenic upflow reactor*. Process Biochemistry, 38(3): p.447-454.
- Zinatizadeh, A.A.L., Salamatina, B., Zinatizadeh, S.L., Mohamed. A.R. and Hasnain Isa, M., (2007), *Palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor*, International Journal of Environmental Research, 1(3): p.264–271.